

### REMARKS

The Office Action mailed December 16, 2003 has been reviewed and carefully considered. Claims 3-13 have been canceled. Claims 14, 22 and 23 have been amended. Claims 14-22 and 24-27 remain pending in this application, with claim 14 being the only independent claims.

Reconsideration of the above-identified application, as amended, and in view of the following remarks is respectfully requested.

In the present office action, the Examiner rejects claims 3-6, 8, 9-11, 13-18, 20, 21-23 and 25 under 35 U.S.C. 102(b) as being anticipated by Aslund et al.

Further, the Examiner rejects claims 7, 12, 19, and 24 -27 under 35 U.S.C. 103(a) as being obvious over Aslund.

Applicants had previously submitted a summary of the invention. Applicants respectfully submit that the present invention is clearly distinguishable from the disclosure of Aslund. It appears that the concept "simultaneous" has been used by Aslund differently as applicants have.

Aslund refers to a simultaneous excitation of the fluorophores using continuous, time-modulated beams. However, in the present application, the excitation is non-simultaneous using an impulse laser as an excitation source for excitation of the fluorophores one after the other, i.e. Applicant utilizes a sequential excitation. The present invention discloses a method, where a simultaneous measurement of the excitation and the decay time of the fluorescences is performed. The excitation process itself is non-simultaneous in the present invention, but rather sequential.

Aslund discloses "a quantitative fluorometer for multiple fluorophores having dual time-modulated beams of excitation light" (see, for example, Abstract, lines 1-2). In Column 1, lines 63-65, Aslund states : "When a fluorophore is excited by light having a sinusoidally modulated intensity, the fluorescence emitted is also sinusoidal modulated." This has been achieved in a microfluorometer which simultaneously excites a plurality of fluorescent targets, Column 3, lines 30-32). The intensity of the excitation at each wavelength is time-modulated at a separate frequency, Column 3, lines 42-43. Further, Aslund discloses that "in the preferred embodiment, light sources 12 and 14 are separate diode lasers, although any source of electromagnetic radiation capable of being time-modulated in intensity may be used. The intensity light source 12 is controlled by a modulator

16 operating at a sinusoidal frequency  $\nu_1$ . In similar fashion, light source 14 is controlled by a second modulator 18 operating at frequency  $\nu_2$ " (Column 5, lines 14-21). "Likewise, the combination of light source 14 and modulator 18 may be implemented by a continuous gas laser." (Column 5, lines 26-28). Even claim 1 of Aslund includes "means for modulating each of said beams at separate modulation frequencies to give each of said beams a specific time modulated waveform".

Hence, the fluorometer as disclosed by Aslund et al. can be described as being based on simultaneous excitation with sinusoidal time-modulation of continuous beams. The time-modulation of the excitation ensures a time-modulation of the answer from the sample and is the basis for a distinction of the fluorophore answers.

In contrast, the present invention discloses an "impulse laser 1 as an excitation source" (see specification published as US 2002/0052048 A1 [0039]). The excitation process is not continuous and not sinusoidally time-modulated, but instead in impulse form. The distinction of the fluorophore excitation and their answers is based on delaying each excitation process of a fluorophore differently, by means of an optical delay 4. The excitation beam is delayed in such a way that only one fluorescence coloring material contained in the sample is always excited near its absorption maximum. By means of the delay, it must be ensured that the individual fluorescence coloring material is thoroughly decayed up to the next excitation process" (published specification US 2002/0052048 A1 [0039]).

Hence the excitation process itself in the present invention is sequential and non-simultaneous in contrast to the Aslund invention. Modulation means, as disclosed by Aslund, are not necessary for the present invention. In addition, sinusoidally time-modulated excitation beams as excitation sources are not subject of the present invention. The decay time is simultaneously measured along with the sequential excitation, but the excitation process itself is non-simultaneous. The fluorescences are excited one after the other (US 2002/0052048 A1 [0020]) and not simultaneously as disclosed by Aslund. This is a profound difference. Aslund has to separate simultaneous excitations, the present invention however ensures that the excitations are decayed before the next excitation takes place. This results in higher measurement sensitivity.

Not only the excitation processes – continuous versus impulse-like excitation – are profoundly different, also the detection and separation process is clearly distinct.

Aslund states that "each beam is synchronized with a separate detector and lock-in amplifier. The fluorophores are simultaneously excited and the combined fluorescent emission is resolved into components corresponding to each fluorophore" (see Abstract, lines 1-7). "A frequency-locked amplifier synchronized to a corresponding modulation frequency" (Column 3, lines 42-45). "Lock-in amplifier 30 is synchronized with the modulation frequency imposed on light source 12 by modulator 16. Similarly, lock-in amplifier 32 is synchronized with the modulation frequency imposed on light source 14 by modulator 18" (Column 6, lines 1-5). Aslund includes "demodulation means for extracting from each of said detected spectral parts of the combined emitted radiation from multiple fluorescent targets the contributions corresponding to each of said targets" (Column 9, lines 29-32).

The separation of the different fluorophore answers is therefore based on modulating the detector with the same frequency as the respective excitations.

In the present invention the separation is done by electronic gates. Lock-in amplifiers would not be able to measure the disclosed separate fluorescence signals of the present invention. "The fluorescence signals are conducted to an optical detector 9 ... In the time range, the detector 9 must be able to approximately follow the fluorescence signals ... The electric signal of the detector 9 is subsequently an image of the excited fluorescence in the time range. A gated integrator 10, with a gate time significantly shorter than the life duration of the fluorescence (e.g., 1 ns), can scan the signal at a defined point in time" (see US 2002/0052048 A1 [0040]).

Therefore, in the present invention the separation of the signals is based on a displacement of electronic gates in the nanosecond range along a time axis (Fig. 2).

The present invention includes all advantages of non-simultaneous detection methods with respect to the sensitivity of the measurement in contrast to simultaneous excitations. It also covers the advantages of a simultaneous measurement system with respect to the measurement time due to its short time range between sub-nanoseconds up to milliseconds. No microscopic or macroscopic probe would be able to experience any relevant shift in location within the short time frame of the multi-fluorescence detection. Therefore, the present invention provides a method for a detection carried out on dead as well as living tissue. The short time frame is achieved by taking advantage of the known life durations of the individual fluorescence coloring materials. The delays between the

different excitations are being fixed in such a manner that they correspond to these life durations. Therefore no time is wasted between the different excitations and an instant measurement is ensured.

In order to further emphasize the differences between the simultaneous measurement of Aslund and the non-simultaneous excitations of the present invention, Applicants amended claim 14. Support for the amendment in claim 14 is disclosed on page 2 and 3 of US 2002/0052048 A1 [0039-0040]. Two features of amended claim 14 are of particular importance to the above discussion, i.e. that an impulse laser (1) is applied as an excitation source and that the excitation processes of the individual fluorescence materials are performed sequentially, one after the other.

Concerning the rejected claims, we submit that the rejections should be withdrawn for the above-mentioned reasons.

For the foregoing reasons applicants submit that independent claim 14 is patentable over the cited reference of Aslund. Dependent claims 15-22 and 24-27 depend directly or indirectly from independent claim 14 and thus, are patentable for the same reasons as independent claim 14.

Applicants submit that the amendments to the claims do not raise new issues that would require further consideration and/or search. Entry of the Amendment and passage of this case to issue are earnestly solicited. However, if for any reason, the Examiner should deem this application not to be in condition for allowance, it is respectfully requested that he telephone the undersigned attorney at the number listed below.

If any additional fees or charges are required at this time in connection with the application, authorization is hereby given to charge our Patent and Trademark Office Deposit Account No. 14-1263.

Respectfully submitted,



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